Implications of Epithelial–Stromal Interaction 1 in Diseases Associated with Inflammatory Signaling

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ABSTRACT: Epithelial–stromal interaction 1 (EPSTI1) was initially identified as an induced gene in breast cancer epithelial cells by cocultured stromal fibroblasts. This discovery led to further investigation and understanding of the role of EPSTI1 in cancer. Aberrant elevation of EPSTI1 occurs primarily in invasive breast cancer epithelial cells. Forced overexpression of EPSTI1 in noninvasive cancer cells can substitute for the stromal fibroblasts. EPSTI1 was further implicated in cancer by our most recent study that identified it as one of the few most upregulated genes in human breast cancer by Krüppel-like factor 8 (KLF8), a pro-cancerous transcription factor in many cancer types. Our study also demonstrated that EPSTI1 interacts with valosin-containing protein to promote the degradation of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) inhibitor alpha, leading to the activation and nuclear translocation of NF-κB. Additionally, EPSTI1 was shown to inhibit apoptosis by inactivating caspase 8. Studies on hepatitis C and E viruses have indicated that EPSTI1 plays a role in inhibition of the viral replication by promoting the expression of protein kinase R or protein kinase RNA-activated, a viral response gene, suggesting a role of EPSTI1 in immune response. Interestingly, in addition to transducing stromal signals, EPSTI1 has been implicated in immune privilege and autoimmune diseases, including systemic lupus erythematosus, rheumatoid arthritis, and histiocytic necrotizing lymphadenitis. This review seeks to comb EPSTI1-related studies as it was cloned a dozen years ago with a particular focus on the mechanisms of its regulation and signaling, as well as its potential roles in the diseases.

KEYWORDS: EPSTI1, KLF8, interferon, cytokine, cancer, autoimmune diseases, inflammation, viral infection, apoptosis, energy homeostasis

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Introduction

Epithelial–stromal interaction 1 (EPSTI1) was first discovered and named in 2002 with a three-dimensional tumor environment assay modeling breast cancer.[1] The gene maps to the human chromosome 13q13.3, has no homology to known genes, and encodes a 38-kDa protein of 307 amino acids. The protein of EPSTI1 contains three coiled-coil domains[1] that potentially play a role in mediating interaction with other proteins and membrane localization of EPSTI. Expression of the protein was induced in the human breast cancer cell MCF-7 upon direct contact with tumor-associated myofibroblasts under coculture.[1–3] Then, the normal tissue expression of EPSTI1 was determined with the highest in the spleen, germinal center in lymphatic tissue, small intestine, salivary gland, testes, and placenta that involve high stromal activities.[1,4] These results suggest that EPSTI1 might serve as a link for interaction or communication between nonstromal tissues, including cancer epithelial cells and stromal tissues, as was gene named.[3] However, cellular localization studies indicated that EPSTI1 appears to be neither a secreted nor a transmembrane protein. Instead, it is a cytoplasmic protein with a nuclear translocation potential.[1,5] Therefore, some extracellular signaling proteins linking the stromal cells and nonstromal cells appear to be needed for EPSTI1 induction. Sequence homology search indicates a highly conserved identity for EPSTI1 across the species,[6] indicating a potentially critical role for EPSTI1 throughout evolution.

In addition to its role in cancer, EPSTI1 has also been implicated in immune privilege,[1,7,8] autoimmune disorders,[9,10] fetal development,[11,12] apoptosis regulation,[13] adipose tissue metabolism,[14,15] and hepatitis virus infection.[16,17] Importantly, all these biological processes appear to be associated with the stromal or inflammatory factors, such as interferon alpha (IFN-α) and transforming growth factor beta (TGF-β). Potential roles for EPSTI1 in the diseases are outlined in Figure 1 with more details described as follows.

Role of EPSTI1 in Cancer Malignancy

Breast cancer is a heterogeneous disease with a large number of genetic alterations. For this reason, six subtypes have been identified, such as luminal A, luminal B, tumor enriched with human epidermal growth factor receptor 2 (HER-2), basal-like, normal-like, and claudin-low subtypes.[18–21] The gene expression profiles and the phenotypes are distinct between different subtypes. For example, luminal A is characterized by the expressions of estrogen receptor (ER),

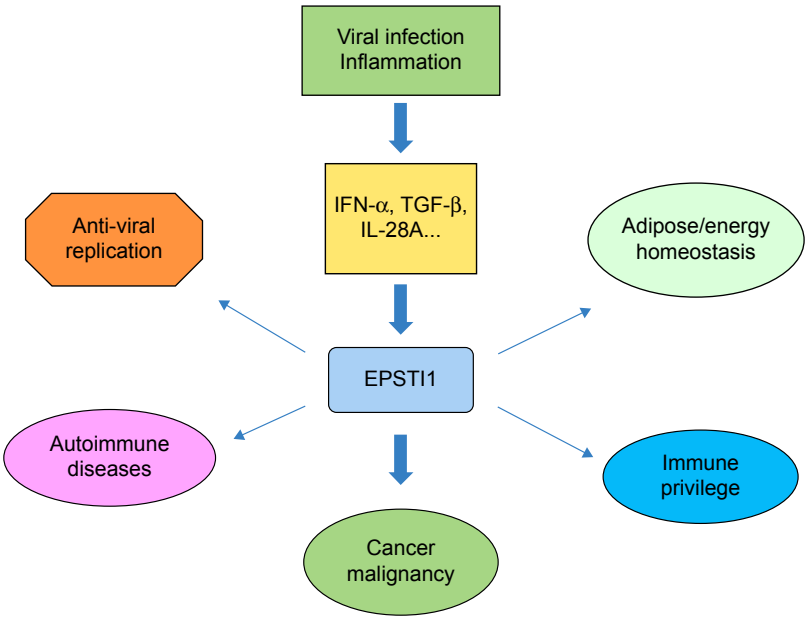

Figure 1. Potential roles of EPSTI1 in diseases. EPSTI1 is induced by paracrine inflammatory cytokines and subsequently links the stromal signals to the indicated various diseases or disorders associated with immune responses.

progesterone receptor (PR), and Bcl-2 and the absence of HER-2. This subtype accounts for up to 60% of the all breast cancer cases.[19,20] The luminal B subtype is characterized by the expressions of ER and PR and the absence of HER-2. Effective treatment for different subtypes of breast cancer relies on understanding the still poorly defined molecular mechanisms underlying the heterogeneity and pathogenesis. EPSTI1 expression was reported to be primarily in basal-like and luminal B subtypes and lower in differentiated subtypes, such as luminal A.[4] The dissemination of tumor cells is normally facilitated by tumor-activated stroma. Forced overexpression of EPSTI1 in the tumor cells abolishes the need for the stromal facilitation.[4] The EPSTI1 association with tumor metastatic potential is further supported by the evidence that EPSTI1 is highly upregulated in many invasive breast cancer cell lines, including HS578T, MDA-MB-231, and BT549, while barely detectable in MCF-7, a nonmetastatic cancer cell line, and MCF-10A, a immortalized nontumorigenic breast epithelial cell line. An analysis of breast cancer tissue samples also indicates that EPSTI1 has a role in metastasis. More than 77% of invasive tumors display high levels of EPSTI1, while only 20% of noninvasive tumors express comparable levels.[4] Further evidence came from two other groups showing that in >300 breast cancer tumor specimens, the aberrant high expression of EPSTI1 is significantly correlated with the metastatic potential and poor patient survival.[22,23] The elevation of EPSTI1 in the tumor cells appears to be promoted by paracrine interferon stimulation and mediated by intracellular interferon response genes, such as signal transducer and activator of transcription 1 (STAT1)[22] and Krüppel-like factor 8 (KLF8).[23]

The epithelial–mesenchymal transition (EMT) is essential for physiological processes, such as embryogenesis and wound healing. However, it also plays a critical role in the initiation of tumor metastasis and induction and maintenance of cancer stem cells.[24–29] Functional studies revealed that EPSTI1 expression in the MCF-7 cells leads to EMT characteristics of a reduction in the epithelial marker protein claudin 1 and an increase in the mesenchymal marker proteins fibronectin and α2β1 integrin and cancer stem cell properties, such as self-renewal and differentiation.[4] In addition to breast cancer, EPSTI1 was shown to play a role in other cancer types, such as human ovarian cancer stem cells[30] as well as human embryonic stem cells,[31] in response to inflammatory cytokines, such as IFN-α and TGF-β.

The implication of EPSTI1 in cancer was bolstered by the study on KLF8 (Fig. 2), a dual transcription factor implicated in cancer of many types[29,32–51] that promotes the expression of EPSTI1 by transcriptional activation of the gene promoter.[23] Notably, KLF8 has been demonstrated to potently induce EMT by repressing E-cadherin[45] and cancer stem cell traits.[29] Importantly, KLF8 is induced by TGF-β, an EMT-inducing stromal factor.[45,52] Co-immunoprecipitation coupled with mass spectrometry identified the interaction of EPSTI1 with valosin-containing protein (VCP). This interaction leads to sequestration and degradation of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) inhibitor alpha (IκBα) and consequent activation of the transcription factor NF-κB (Fig. 2).[23] This is of interest as NF-κB targets many pro-cancerous genes and plays a vital role in immune response as well.[53] Our study also showed that ectopic overexpression of EPSTI1 in the two noninvasive cell lines, MCF-7 and

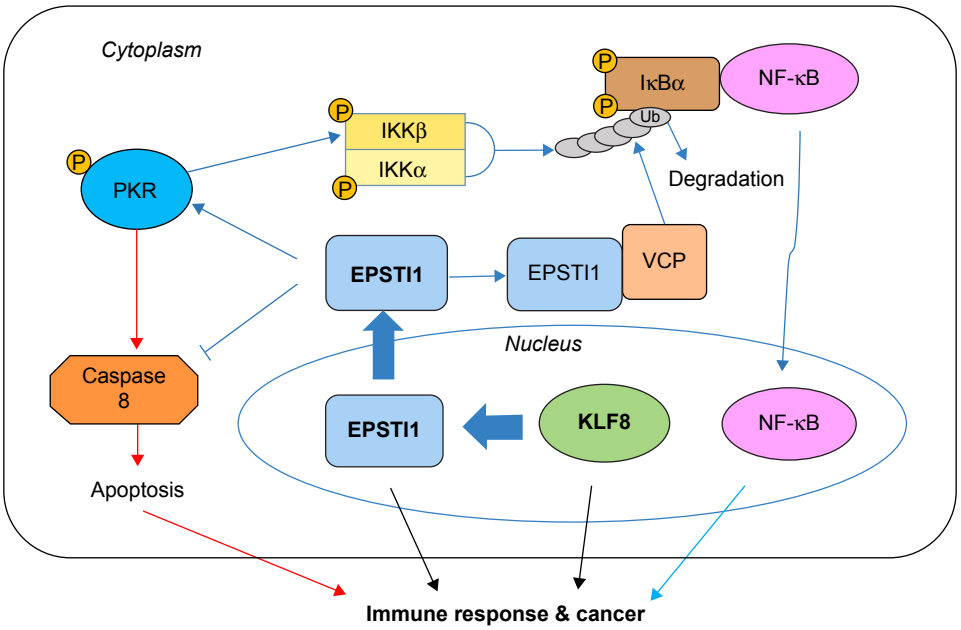

Figure 2. Mechanisms of EPSTI1 regulation and signaling. EPSTI1 transcription is turned on by KLF8. In the cytoplasm, EPSTI1 promotes the activation of NF-κB by PKR phosphorylation of (IκB kinase) IKK and VCP-mediated degradation of IκBα. On the other hand, EPSTI1 inhibits apoptosis by inactivating caspase 8. The overall outcomes favor the disease progression.

MCF-10A, was sufficient to promote cell invasion. Xenograft experiments showed that inducible overexpression of EPSTI1 in the MCF-7 cells primarily enhances the cell invasion rather than proliferation, leading to malignant growth of primary tumors and metastases.[23]

Cancer is regulated by the loss of various fundamental balances, including that between cell proliferation and apoptosis. EPSTI1 has been shown to interact with caspase 8 to inhibit apoptosis in breast cancer cells (Fig. 2).[13] Silencing EPSTI1 in the cells results in a significant increase in cell apoptosis. Being consistent with the change in the activity of caspase 8, the manipulation of EPSTI1 expression greatly impacts the cleavage of poly(ADP-ribose) polymerase 1 (PARP-1), a hallmark of active apoptosis.[37,54,55] In concert with these findings, some interesting connections can be drawn between the pathways associated with EPSTI1. Primarily, both EPSTI1's upstream KLF8 and downstream NF-κB promote the expression of matrix metalloproteinase 9 (MMP9) and cyclin D1, which are important for cancer invasion and proliferation, respectively, by activating the transcription of the genes in a p300-dependent fashion.[41,42,56,57] EPSTI1 inhibition of PARP-1 cleavage is also of interest, given that the nuclear presence of KLF8 depends on its interaction with PARP-1.[37] Notably, PARP-1 binds to p300 to mediate the NF-κB transcriptional activation.[58] However, PARP-1 does not exist with KLF8-p300 complex at the cyclin D1 gene promoter. This revokes the possibility that KLF8 and NF-κB work synergistically to activate their targets, such as cyclin D1 and MMP9.[37] In addition to inhibiting caspase 8, EPSTI1 was also shown to interact with RAC-alpha serine/threonine-protein kinase (AKT1) and breast cancer antiestrogen resistance (BCAR3) protein.[13] AKT is a master player in the cell survival pathways, and BCAR3 plays a role in breast cancer resistance against estrogen-based hormonal therapy. Taken together, these data indicate that EPSTI1 inhibits apoptosis potentially by complex mechanisms to support cancer cell survival.

Role of EPSTI1 in Immune Response and Viral Infection

Recent evidence has built on the role of EPSTI1 in the immune response. Several reports have indicated that EPSTI1 is one of the major target genes for INF-α in cancer cells.[4,6,9,22] It was further demonstrated that EPSTI1 is upregulated in hepatoma cells in response to hepatitis C virus (HCV) or hepatitis E virus infection.[5,16] Specifically, when EPSTI1 expression is stimulated by IL-28A, a type III interferon, the HCV replication is inhibited in Huh 7.5 cells, a hepatoma-derived highly HCV-permissive cell line. Treatment with IL-28 and IFN-α has a synergistic effect on EPSTI1 expression and inhibition of the virus replication and viral clearance. Mechanistically, EPSTI1 represses HCV replication by increasing the expressions of protein kinase R or protein kinase RNA-activated (PKR), 2′-5′-oligoadenylate synthetase 1 (OAS1), and ribonuclease L (RNase L), three well-known antiviral genes. OAS1 and RNase L expression levels were reversed upon PKR knockdown, thus illuminating the importance of the double-stranded RNA response PKR.[59] Silencing the IFN-α/IFN beta receptor 1 leads to a decrease in PKR mRNA expression, which can be rescued by overexpressing EPSTI1. This result suggests a role of EPSTI1

downstream of the interferons in promoting PKR expression. Indeed, promoter luciferase reporter assay showed that EPSTI1 overexpression results in the activation of PKR gene promoter.[5] Whether EPSTI1 acts directly or indirectly on the PKR gene promoter remains to be determined. Nevertheless, EPSTI1 stimulation of PKR expression appears to be important. It is known that PKR can stimulate NF-κB activation via binding and activating IκB kinase.[60] Indeed, knockdown of PKR in Huh 7.5.1 cells causes a decrease in IFN-β response and NF-κB activation.[52] In addition to expression, phosphorylation of PKR was also greatly increased upon EPSTI1 overexpression.[5] Once phosphorylated, PKR becomes activated to catalyze phosphorylation of eIF2alpha, resulting in an inhibition of protein synthesis, increase in autophagy, and stimulation of apoptosis.[60–62] This is of interest in that EPSTI1 inhibits the apoptosis-promoting functional arm of PKR leaving the NF-κB-activating functional arm active to ultimately tilt the balance toward favoring survival and proliferation of tumor cells.[13] The stimulation of PKR expression by EPSTI1 allows further phosphorylation of IκBα and subsequent EPSTI1-facilitated degradation of IκBα to ensure the downstream activation of NF-κB (Fig. 2).

The role of EPSTI1 in immune response is quite new, but novelty has already arisen around many aspects of its genesis. HCV stimulation of EPSTI1 in the Huh 7.5 cells provides insight into its possible mechanisms. The HCV nonstructural protein 3 was shown to enhance the hepatoma cell invasion through NF-κB-mediated activation of MMP9 along with the activation of extracellular signal-regulated kinases (ERK1/2) and p38 MAP kinase.[17] These findings close the gap on how HCV infections can promote tumorigenesis, in which EPSTI1 may be involved. The molecular background of EPSTI1 further intertwines cancer with the immune response. IL-28A can stimulate migration of the HT1376 and T-24 bladder cancer cell lines via NF-κB-mediated MMP9 expression. This stimulation again involves p38 and ERK1/2.[63] These findings are apparently controversial to the role of EPSTI1 in hepatitis virus clearance described earlier. However, it is conceivable that EPSTI1 may play a role in switching the viruses from virulent to temperate status to favor the neoplastic role of the viruses. Alternatively, the battle between the virus and EPSTI1 leads to chronic inflammation that consequently potentiates the tumorigenesis and malignancy.

Potential Role of EPSTI1 in Autoimmune Disorders

The immune system is a system of checks and balances both on the molecular and cellular levels. Autoimmune diseases are the result of a disruption of these checks and balances. The disruption can happen in various independent ways, as is apparent by various autoimmune diseases afflicting the same individual patient.[64] Few effective treatments and no cures exist for many autoimmune diseases.[65]

EPSTI1 may be a vital piece in developing such therapies. It was found upregulated in many of the most serious autoimmune diseases, including systemic lupus erythematous (SLE),[9] Sjogren's syndrome,[66] spondyloarthritis,[67] idiopathic thrombocytopenic purpura,[9] histiocytic necrotizing lymphadenitis,[68] and rheumatoid arthritis.[10] In a paper showing the gene expression profile of SLE, EPSTI1 was found upregulated in B lymphocytes and cytotoxic ($CD8^+$) T lymphocytes, while the highest expression of EPSTI1 was in monocytes.[9] In addition, EPSTI1 is upregulated in histiocytic necrotizing lymphadenitis, a rare disease characterized by an excess mass primarily filled with macrophages, dendritic cells, and plasmacytoid cells.[68] Consistently, EPSTI1 upregulation was considered to be a predictor of nonresponse in rituximab that targets $CD20^+$ B cells for rheumatoid arthritis treatment.[10] Taken together, these findings bring to light that EPSTI1 could play a function in autoimmune diseases through various types of immunocytes. Understanding the molecular functions of EPSTI1 in these cells could shed new light on the pathogenesis of the diseases.

This is complicated by the findings that EPSTI1 is highly upregulated in the immune privilege sites, such as the placenta and testis. In these sites, inflammation is physiologically dampened even in the presence of invading antigens.[1,7,8] Interestingly, the important cytokine for immune privilege TGF-β[69] has been shown to stimulate the expression of upstream KLF8.[45,70,71] It would be interesting to test if EPSTI1 plays a role in immune dampening cell types, such as regulatory T lymphocytes and M2 macrophages, prevalent in immune privilege sites as well as malignant tumors (or pathological immune-privileged sites).[72]

The high expression levels of EPSTI1 in the placenta and testes have drawn attention to how it functions in these sites. EPSTI1 was reported overexpressed in an in vitro model of maternal environment represented by the coculture of bovine embryos with oviduct epithelial cells.[11] Notably, this study also found PKR to be upregulated and to presumably serve as a feedback mechanism to halt STAT1-dependent production of the other interferon response genes. This raises a possible immune dampening feedback mechanism for EPSTI1 as well. EPSTI1 was also reported to play a role in endometrium remodeling during the early phase of pregnancy. Early phase of pregnancy shares many similarities with the initiation of cancer malignancy. Indeed, TGF-β plays an important role in endometrium remodeling and associated MMP production.[12] Upregulation of EPSTI1 in the testes was linked to male fertility.[8] Spurred by this finding, researchers have generated an EPSTI1 knockout rat model, in which EPSTI1 expression in the spermatogonia is specifically ablated using the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 technique.[73] The use of this model will likely shed new lights on the role of EPSTI1 in male fertility and stimulate the generation of EPSTI1 genetically engineered mouse models for further characterization of the protein in vivo.

EPSTI1 could also confer viral resistance to epithelial cells, which would help solve the mystery of how immune-privileged

sites resist infections in the absence of normal inflammatory responses.[4,23] This notion could be supported if EPSTI1 upregulates PKR to not only promote cellular immune response[5] but also ensure immune-privileged sites/cells, where apoptosis needs to be suppressed,[7] which remain unattacked by inactivating caspase 8. Importantly, apoptosis appears to be suppressed in many cases of autoimmune diseases, such as rheumatoid arthritis.[74]

Taken together, the above-described findings raise a possibility that in immune-privileged sites, such as the testes and placenta, EPSTI1 is induced primarily in response to stromal factors, such as IFN-α and TGF-β, released by immune inhibitory cells, such as regulatory T lymphocytes and M2 macrophages, to protect the immune-privileged cells from immune attacks and ensure effective fertility and pregnancy. Failure to maintain the immune privilege leads to autoimmune disorders. Tumors hijack the immune privilege mechanism for developing malignancy to evade immunosurveillance and resist immunotherapy. Interference with EPSTI1's expression, cellular localization, protein interaction, and signaling pathways can be explored for EPSTI1-based immunotherapies against these related diseases.

Potential Role of EPSTI1 in Adipose Metabolism and Energy Homeostasis

Two recent studies have identified EPSTI1 as a new specific marker for the BRITE/beige adipose tissue, also known as brown-in-white adipose tissue.[14,15] One of the studies also claims that human brown adipose tissue abundantly expresses genes unique to BRITE/beige cells, while lacking the classical brown adipocyte-selective genes. The brown fat tissue primarily functions for energy consumption, whereas white adipose tissue mainly works to store energy.[75] Although research on EPSTI1 in adipose tissue has just been kick-started and the area remains in its very infancy, the results nevertheless suggest a potentially important role of EPSTI1 in energy expenditure associated with lipid metabolism and energy homeostasis, which is worth further investigation.

Conclusion

Significant progress in EPSTI1 research has been made since it was first reported in 2002. Although EPSTI1 is now known in more detail in regulating cancer than immune functions and lipid homeostasis, inflammatory cytokines and stromal factors appear to be the keys that relay the stromal messages to the cells affected where EPSTI1 is induced. KLF8 appears to be a critical signaling mediator downstream of these factors and immediately upstream of EPSTI1. The studies also suggest that all the disorders listed in Figure 1 seem to arise when the regulation of immune functions by EPSTI1-associated mechanisms goes awry. In addition to immunocytes, tumor-associated nonimmunocytes, such as myofibroblasts, also play a critical role in inducing EPSTI1 expression via releasing paracrine inflammatory cytokines in cancer malignancy. The quick emerging of the EPSTI1 research field offers new opportunities to further investigate the mechanistic details regarding this protein, such as its posttranslational modifications, intracellular shuttling, and interaction partner molecules. Understanding these mechanisms could help pave the way toward developing new therapeutics against these diseases/disorders.

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Author Contributions

Wrote the first draft of the manuscript: JG. Made critical revisions and approved final version: JZ. All authors reviewed and approved of the final manuscript.

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